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Metallothionein Synthesis and Degradation: Relationship to Cadmium Metabolism

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Metallothionein is an integral component of the mechanism that regulates the metabolism of cadmium and zinc. The synthesis of this protein can be "induced" by oral or parenteral administration of either metal. The metallothionein mRNA content of liver polysomes is increased shortly after an influx of small amounts of either metal into hepatocytes. After sufficient amounts of this poly (A+) RNA have been synthesized, there is a concomitant increase in metallothionein biosynthesis and metal binding. Unlike synthesis, the degradation of metallothionein is markedly influenced by the species of metal bound. By using *in vivo* and *in vitro* techniques, it has been possible to demonstrate that resistance of metallothionein to degradation follows the order: thionein < zinc metallothionein < cadmium metallothionein. Moreover, while the polypeptide chains of cadmium metallothionein are degraded, it appears that liberated cadmium ions are quickly incorporated into nascent chains of thionein. The latter explains why the cadmium content of liver and kidney increases with age and environmental exposure. Since both zinc and cadmium bind to metallothionein, it appears that the binding sites provided by this inducible species provide a locus for interaction between zinc, a nutrient, and cadmium, an environmental contaminant.

Introduction

Cadmium and zinc are both group IIB elements, therefore, it is understandable that the metabolism of both is similar. Since research in the area of a cadmium–zinc interaction has been well reviewed (*1*), this review will be primarily limited to experiments conducted in the author's laboratory.

In our initial experiments, we were interested in studying the influence of dietary cadmium on various biochemical parameters in swine (*2*). It was clear that cadmium retention was closely related to the dietary cadmium content. As had been noted by others previously (*1*), we found that the liver and kidney were the primary sites of dietary cadmium deposition. Virtually no cadmium was found in muscle, bone or blood.

Gel filtration chromatography of 40,000*g* supernatants of kidney homogenates demonstrated that the majority of the soluble, intracellular cadmium was associated with a low molecular weight fraction. Subsequent chromatographic steps allowed us to establish that this cadmium-binding fraction was renal metallothionein (*3*). Its properties were similar to equine and human metallothioneins isolated previously (*4*, *5*). We also were able to detect a cadmium-binding metallothionein in chicks fed 75 ppm cadmium (*6*).

It was clear from this early work that feeding cadmium resulted in a concomitant increase in the zinc content of liver and kidney. Most of this increase in tissue zinc was accounted for as the soluble, low molecular weight species, metallothionein. These data were strongly suggestive of a link between cadmium and zinc metabolism that involves metallothionein which acts as a common binding ligand for both metals.

Metallothionein Synthesis

Metallothionein was not detected in appreciable concentrations in animals not fed cadmium. Therefore, a series of experiments was undertaken to establish if this protein was induced by cadmium. We found that when cadmium containing ^{115m}Cd was injected simultaneously with ^{3}H-cystine, substantial amounts of radioactivity were incorporated into

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metallothionein in liver and kidney (*7*). When the rats were injected with only tracer amounts of cadmium, no "induction" of metallothionein occurred. Moreover, when actinomycin D, an inhibitor of mRNA synthesis, was administered prior to cadmium, the induction of metallothionein was not observed. These data led us to propose that metallothionein is indeed an induced protein. It was also possible to show that a relatively small dose of cadmium, which could induce metallothionein, seemed to protect rats from an orally administered acute dose of the metal (*8*). This observation was in line with the concept that metallothionein is in some way able to act as a sequestering agent which can protect the body from the toxic effects of cadmium (*1*).

Since the body burden of cadmium is usually extremely low, it is highly unlikely that metallothionein exists for the sole purpose of sequestering cadmium ions. With that rationale we set forth to examine the role of metallothionein in mammalian zinc metabolism. It was possible to demonstrate that metallothionein could be induced by zinc just as it could by administration of cadmium. Specifically zinc (^{65}Zn) was incorporated with C^{14}-cystine during the induction of metallothionein in rat liver (*9*). Intraperitoneal zinc administration resulted in an increase in the serum zinc concentration for a 4–12 hr period. As zinc was cleared from the plasma, it appeared to be taken up to a large extent by the liver. Most of this increase in intracellular hepatic zinc was accounted for in the cytosol fraction as metallothionein-associated metal (*10*). The increase in the hepatic zinc content and metallothionein-associated zinc was inhibitable by administration of actinomycin D prior to the zinc. This suggests that RNA and protein synthesis are required at some phase of the zinc accumulation process in hepatocytes.

Zinc accumulation following an intraperitoneal dose seemed to reach a maximum by 24 hr after administration. Therefore, we conducted experiments to investigate the rate of hepatic metallothionein synthesis for up to 24 hr after administration of zinc. We found that the rate of synthesis, as measured by ^{35}S-cystine incorporation, increased to a maximum by 5 hr after zinc. This subsequently returned to a basal level by 20 hr post-administration (*11*). In contrast to the rate of ^{35}S-cystine incorporation, zinc incorporation into metallothionein was only maximal by 20 hr after zinc administration. This zinc stimulated increase in the rate of metallothionein synthesis could be blocked by prior administration of actinomycin D, cordycepin, or cycloheximide. These results suggest that accumulation of significant amounts of zinc is directly correlated with an increase in the rate of metallothionein biosynthesis.

Inhibition of the synthesis of metallothionein by two different inhibitors of transcription strongly suggests that the induction of metallothionein occurs via a mechanism that involves stimulation of the transcription of metallothionein mRNA. In order to gain more information about this mechanism, we isolated rat liver polysomes and translated their mRNA in a cell-free, polysomal system (*12*). Polysomes from zinc-injected animals synthesized more metallothionein-like polypeptides than polysomes from control rats. In the next phase of these experiments, it was possible to show that the actual amount of metallothionein mRNA present in polysomes, after zinc administration, follows a time course that is nearly identical with that seen for the rate of metallothionein synthesis *in vivo* (*13*). For these latter experiments, poly(A+) mRNA was isolated from rat liver polysomes by oligo-(dT) cellulose chromatography. The poly(A)-containing mRNA was translated in a wheat germ, cell-free synthesizing system containing ^{3}H-glycine, lysine, and serine. The amount of newly translated ^{3}H-metallothionein could be quantitated either by gel filtration or activated thiol-Sepharose chromatography. The amount of polysomal metallothionein mRNA was maximal 5 hr after administration of zinc. This stimulation in metallothionein message was inhibitable by prior administration of actinomycin D. The experiments cited above, as well as experiments from other laboratories, fairly conclusively establish that liver metallothionein is an induced protein. The induction process appears to require an increase in the amount of intracellular metal. In view of the important roles that zinc has for maintenance of cellular processes, as well as the marked cytotoxic properties of cadmium, it is not surprising that an intracellular ligand with strong binding properties evolved.

Metallothionein Degradation

Hepatic metallothionein does not serve a terminal storage function, since it is capable of undergoing degradation. In an attempt to investigate this aspect of metallothionein biochemistry, we induced a zinc metallothionein, i.e. a metallothionein which contained nearly exclusively zinc as the bound metal, that was labeled with ^{65}Zn and ^{35}S-cysteine. The degradation of this labeled protein was followed after it reached its maximal level of incorporation of these labeled precursors, i.e., 24 hr after zinc administration (*14*). We found that hepatic zinc metallothionein was fairly rapidly degraded. The half-life ($t_{\frac{1}{2}}$) for Zn^{65} and S^{35} in the $t_{\frac{1}{2}}$ protein was 18

and 20 hr, respectively. The marked similarity in $t_{\frac{1}{2}}$ suggests that the polypeptide chain is degraded simultaneously with or shortly after removal of bound zinc.

The degradative behavior of induced cadmium metallothionein is markedly different from that observed when zinc is the metal bound to the thionein polypeptide chain. Hepatic cadmium metallothionein was induced by cadmium and labeled with ^{115m}Cd and ^{3}H-cystine. Maximal labeling was obtained at 24 hr after cadmium administration. The $t_{\frac{1}{2}}$ of ^{3}H in cadmium metallothionein was 3.5 days (*15*). Of paramount importance, from the standpoint of cadmium toxicity, was the observation in these studies that cadmium (^{115m}Cd) did not appear to be removed from the protein, at least during the 9 days that degradation was monitored. These data suggest that, *in vivo*, the binding affinity of cadmium to metallothionein, relative to zinc, is sufficient to allow for a preferential binding of cadmium to newly synthesized metallothionein polypeptides. This would have the effect of maintaining the cadmium content of metallothionein with concomitant continual degradation of the polypeptide chains.

In an effort to gain insight into the difference in proteolytic susceptibility of zinc- and cadmium metallothioneins, we conducted *in vitro* experiments with both forms of the protein as well as with thionein, the metal-free form of the protein (*16*). Each form was degraded by incubation with trypsin and pronase, while chymotrypsin had no influence on the degradation of any form of the protein. Lysosomal proteases were extremely active in degrading each form of the protein to the constituent amino acids. Moreover, metal binding had a pronounced influence on the rate of proteolysis. At a substrate to enzyme ratio of 300:1 ^{35}S-thionein was more than 80% degraded in about 1.5 hr, whereas about 3 hr was required to degrade zinc metallothionein to that extent. In contrast, cadmium metallothionein was only 70% degraded after an incubation period of 24 hr. These data show that differences in susceptibility to proteolysis have a marked influence on the metal bound to metallothionein within cells. Since thionein is rapidly degraded, cellular concentrations of this species do not accumulate. However, since cadmium metallothionein is extremely resistant to degradation, its concentration in cells is more likely to be maintained (Fig. 1).

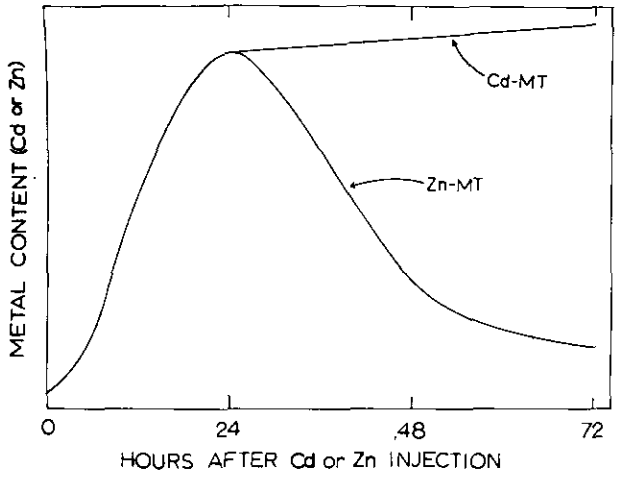

FIGURE 1. Relationship of cadmium and zinc metallothionein synthesis and degradation to hepatic accumulation of injected cadmium and zinc.

Zinc Metabolism and Homeostasis

Another phase of our work was designed to investigate the role of metallothionein in zinc metabolism. Using fluctuations in dietary zinc as a method to alter zinc status, it became clear when zinc status was elevated, the metallothionein content was increased in both liver and intestinal mucosa (*10, 17, 18*). The intestinal zinc-binding protein (*19*) has all of the characteristics necessary for classification as a metallothionein (*4*). The elevation of zinc status was concomitant with a decrease in intestinal zinc absorption and sequestration of intracellular mucosal cell zinc as metallothionein (*10, 18, 19*). Actinomycin D completely abolished the ability of animals to make intestinal metallothionein and to regulate intestinal zinc absorption, strongly suggesting that the two processes are linked. It is possible to show that during chronic cadmium toxicity, mucosal cell cadmium is associated with intestinal metallothionein (*20*). Cadmium has been shown to replace zinc in metallothionein (*4*). Therefore, it is likely that, in attempting to fulfill its role in the homeostatic regulation of zinc metabolism, intestinal metallothionein is able to prevent substantial amounts of cadmium from traversing the intestinal mucosal cells to the systemic circulation.

A scenario of events can be envisioned starting with the absorption of zinc which illustrates how metallothionein plays a role in the overall metabolism of this essential trace element. When zinc enters the intestinal lumen, it is rapidly transported across the mucosal cells to the basolateral membrane for uptake into the plasma. This elevates the plasma zinc concentration, which acts as a signal to bring about the induction of hepatic and intestinal metallothionein mRNA, which in turn is encoded into metallothionein polypeptides. In the intestine,

nascent thionein polypeptide chains are able to interact with zinc as it enters the mucosal cells from the intestinal lumen. Since the binding of zinc to metallothionein is probably greater than the transport components necessary to remove zinc from the intestinal mucosal cell to the circulation, the metal remains bound to metallothionein. Presumably, dietary cadmium is handled in a similar fashion. In the liver, the nascent thionein polypeptide chains appear to act as a ligand that is involved in the accumulation of zinc within hepatocytes.

Zinc and Cadmium Metabolism in Isolated Hepatocytes

In a recently completed series of experiments with rat liver parenchymal cells in primary culture, we have been able to demonstrate that hepatic zinc metabolism is an extremely dynamic process. When hepatocytes are maintained in primary culture, the zinc influx process occurs concomitantly with an exchange process, thus there is little net accumulation of intracellular zinc (*21*). However, if the zinc concentration of the extracellular environment (medium) is elevated, or if glucocorticoids are present in the medium, net accumulation occurs (*22*). Accumulation is a temperature and energy dependent saturable process. The extra zinc that is accumulated during these two situations is associated with metallothionein, indicating the protein acts as a ligand that is responsible for maintaining intracellular zinc. The metabolism of cadmium in isolated hepatocytes is nearly identical, except that the accumulation phase is very effective, because, unlike zinc, cellular efflux of cadmium proceeds at a slower rate (*23*).

Low Dietary Calcium and Cadmium Metabolism

It is difficult to evaluate the metabolism of any trace element without considering the overall nutritional status of the individuals in question. As part of our experiments on chronic cadmium toxicity, we investigated the role of the dietary calcium level on cadmium retention. It is well known that feeding a low calcium diet will result in an acceleration of calcium absorption by the intestinal mucosal cells (*24*). We provided cadmium in the drinking water for rats at 25 ppm. Those rats fed a low calcium diet accumulated substantially more cadmium than those fed a normal level of calcium (*25, 26*). A similar effect was noted earlier (*27*). During periods of low dietary calcium, substantially more calcium-binding protein (CaBP) is produced in the intestinal mucosal cells. This protein is in some way involved in the absorption of dietary calcium (*28*). It has been possible to show that during periods of calcium restriction, when the levels of this protein are particularly high, cadmium will also bind to the protein. The enhanced cadmium binding activity of CaBP may account for the increased accumulation of the metal during periods of low calcium status (*25, 26*). A summary of the influence a low calcium diet has on cadmium uptake is presented in Figure 2.

In this review, it is not possible to discuss all aspects of cadmium toxicity. However, I should mention the influence cadmium appears to have on the activation of vitamin D to its hormonal form (1,25-dihydroxycholecalciferol). In early experiments we demonstrated that the renal conversion of 25-hydroxycholecalciferol, via the renal enzyme 25-hydroxycholecalciferol-1-hydroxylase was markedly reduced in animals administered substantial amounts of cadmium (*29*). This observation has been substantiated subsequently (*30*). Based upon these observations, it is indeed likely that during periods of chronic cadmium toxicity renal concentrations of cadmium develop that are sufficient to alter the vitamin D hydroxylating capacity of the kidney, which in turn will influence calcium metabolism.

Figure 3 is a schematic representation of how various aspects of cadmium metabolism and toxicity may be related to metallothionein. As cadmium enters the intestinal mucosal cell, it probably helps signal the induction of intestinal metallothionein. This protein in turn sequesters a major portion of the dietary cadmium that is taken up within the mucosal cells. Cadmium that is transported via the plasma is initially taken up by the liver cells and is incorporated into hepatic metallothionein. We have been able to show that hepatic cadmium-thionein is able to undergo degradation, although at a slower

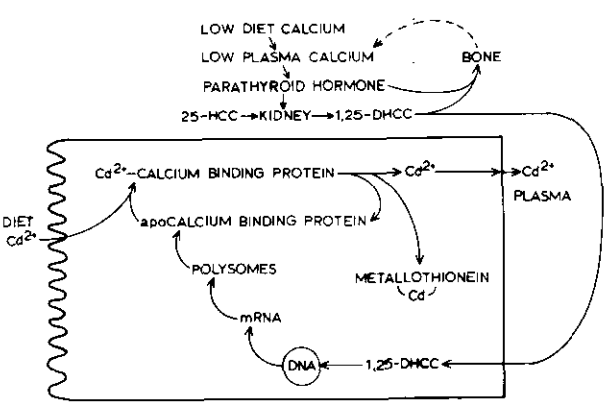

FIGURE 2. Proposed mechanism for the influence of low dietary calcium on enhanced cadmium accumulation.

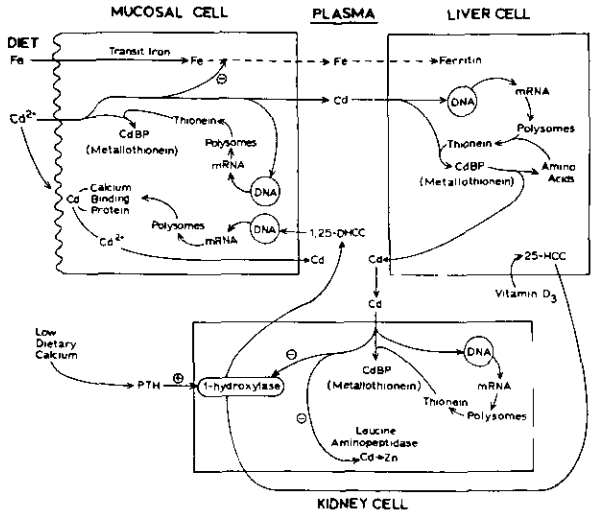

FIGURE 3. Proposed role of metallothionein in cadmium metabolism and toxicity.

rate than that found for either thionein or zinc metallothionein. During this period, cadmium is probably able to interact with other cellular components. Eventually cadmium appears to be deposited in kidney cells. Again most cadmium is bound to the intracellular ligand, metallothionein. Since the kidney cells appear to be able to synthesize metallothionein, it is unlikely that cadmium is transported from hepatocytes to kidney cells as metallothionein. It is hoped that the experiments discussed in this review point out that cadmium toxicity cannot be considered as an isolated situation; rather it must be integrated into all aspects of cell physiology and trace element nutrition and metabolism.

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